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JANUARY 1967

DEPARTMENT OF THE ARMY
Fort Detrick
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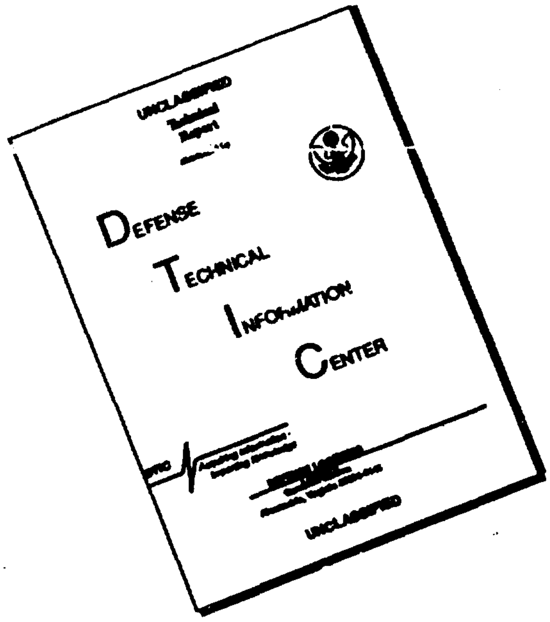

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ABSTRACT

Mutational loss of pesticin I, a bacteriocin-like substance produced by *Pasteurella pestis*, results in concomitant loss of a coagulase and a fibrinolytic factor. No relationship was detected between pesticinogeny and other tested properties either associated with virulence or peculiar to *P. pestis*. Pesticin I was distinguished from the coagulase and fibrinolytic activities on the basis of anatomical distribution, behavior during gel filtration, and sensitivity to heat. Coagulase and the fibrinolytic factor were not differentiated by these criteria. Spontaneous suppressor mutations causing reversion to pesticinogeny were not detected, nor were such mutants obtained by treatment with ultraviolet light or 2-aminopurine. Attempts to demonstrate a common activator of pesticin I, coagulase, or the fibrinolytic factor in extracts of pesticinogenic cells were not successful. These results are in accord with the hypothesis that at least two structural genes for the three activities reside on a replicon distinct from the chromosome proper. Fibrinolytic activity was significantly reduced in the presence of 0.003 M ϵ-aminocaproic acid and was nonexistent on fibrin films freed of endogenous plasminogen by treatment with heat. Fibrinolytic activity on heated films could be restored by addition of plasma or serum from six mammalian species. Accordingly, the plague fibrinolytic factor, like staphylokinase or urokinase, promotes the conversion of plasminogen to plasmin.

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I. INTRODUCTION

Most workers agree that Pasteurella pestis, the causative agent of bubonic plague, and Pasteurella pseudotuberculosis are very closely related species. Nevertheless, these organisms are easily distinguished by the symptoms of the diseases that they cause as well as by their invasiveness or ability to initiate a generalized infection from an intradermal or subcutaneous site of injection. For example, P. pseudotuberculosis most commonly causes a mild and localized mesenteric lymphadenitis in man[1,2] that bears little resemblance to plague, an acute and frequently fatal infection. P. pestis is highly virulent in rats, which are generally resistant to infection with P. pseudotuberculosis. Both species are virulent in guinea pigs provided that the organisms are injected intraperitoneally; only P. pestis can infect this host following abrasion of the skin.[3] The LD_{50} for P. pestis in Swiss-Webster mice (Fort Detrick strain) was less than 10 cells when injected by the intravenous, intraperitoneal, or subcutaneous routes; that for P. pseudotuberculosis approached this value only after intravenous injection.[4] Accordingly, only P. pestis seems capable of consistent initiation of a generalized infection from peripheral sites of injection. This ability probably reflects the fact that P. pestis, but not P. pseudotuberculosis, is infectious by fleabite, the normal process whereby plague is transmitted in nature. Both species can survive in fleas.[3,5] Pseudotuberculosis is probably acquired following ingestion of the organisms. This possibility, however, has not yet received experimental support.[6]

Another difference between the two species is the presence of fibrinolytic[7] and coagulase[8] activities in P. pestis. A correlation between expression of the fibrinolytic factor (F) and coagulase (C) was reported by Domaradskii,[9] who failed to detect either activity in P. pseudotuberculosis. Brubaker, Surgalla, and Beesley[10] subsequently noted that production of F and C is correlated with production of pesticin I (PI), a bacteriocin-like substance active against P. pseudotuberculosis.[11-15] The presence of PI, F, and C in P. pestis might account in part for the general distinctions between plague and pseudotuberculosis. This possibility received support from the finding that lethality in Fort Detrick mice of a non-pesticinogenic strain of P. pestis resembled that of wild-type P. pseudotuberculosis.[4]

This report is concerned with defining the relationships among PI, F, and C with emphasis on their mode of inheritance. A search for other properties associated with pesticinogeny was carried out, and results of a study of the mechanism of fibrinolysis are discussed.

II. MATERIALS AND METHODS

A. STOCK CULTURES

P. pestis strains 019 and 14 were obtained through the kindness of Dr. T.W. Burrows. A second culture of strain 14 was made available by Dr. S. Quan. As these isolates differ in certain respects, they are here designated strains B14 and Q14, respectively. *P. pestis* strain URR was subcultured from a urease-positive variant isolated by Mollaret et al.;[16] the latter was received from Dr. W.D. Lawton. All strains prefixed with the letter G were kindly supplied by Dr. D.M. Eisler.

In order to avoid loss of pesticinogeny or virulence due to shifts of population during long periods of preservation, the following method developed by Dr. W.D. Lawton was used to maintain stock cultures. Approximately 10^{10} viable cells of each strain were suspended in 4 ml of sterile 0.06 M potassium phosphate buffer, pH 7.2 (phosphate buffer), contained within screw-capped tubes. Each tube then received 6 ml of sterile glycerol and, after mixing, was stored at -23 C. The stock cultures remained liquid at this temperature and were used for direct inoculation of agar slopes.

B. MEDIA AND CULTIVATION

Blood agar base (BAB) obtained from the Baltimore Biological Laboratories was used as a general solid medium. Larger numbers of organisms were grown at 26 C in 200 ml of Difco heart infusion broth (HIB) per 2-liter flask. Each flask was inoculated with cells removed from a slope of BAB and incubated overnight on a shaker operating at 90 oscillations per minute through a 3-inch stroke.

C. CELL-FREE EXTRACTS AND CELLULAR DEBRIS

Cells from liquid cultures were harvested by centrifugation at 27,000 x g for 10 minutes at 5 C and were washed twice with phosphate buffer. The cell crop was then suspended in a volume of 0.1 M tris(hydroxymethyl)aminomethane-Cl buffer, pH 7.8 (tris buffer), corresponding to 1% of the original culture volume. After disintegration at 20,000 psi in the French pressure cell, the cell extract was prepared by centrifugation at 27,000 x g for 10 minutes. The pellet was washed five times with phosphate buffer and then resuspended in tris buffer to a volume equivalent to that of the corresponding cell extract. When results are expressed in terms of dry weight, the cellular debris, consisting of large particles and some whole cells, was washed five times with distilled water, lyophilized, and subsequently sterilized with chloroform vapor.

D. FIBRINOLYSIS

Fibrin films were prepared by a modification of the method of Astrup and Mullertz.[17] To a petri dish containing 10 ml of a 0.25% solution of fibrinogen was added with gentle swirling 0.5 ml of sodium borate buffer, pH 7.75 (borate buffer), prepared by the method of Lewis and Ferguson[18] containing 25 NIH units of bovine thrombin. Coagulation was complete in 20 to 60 seconds. Material to be tested for F was diluted in borate buffer, and 0.02-ml samples were applied to the surface of fresh fibrin films. The plates were incubated for 18 hours at 37 C and then observed for fibrinolysis.

To inactivate endogenous plasminogen, fibrin films in flat-bottomed glass petri dishes were incubated for 55 minutes at 70 C on the surface of a shallow water bath.[19] In other experiments, ϵ-aminocaproic acid (EACA) was added to the preparation of fibrinogen followed by coagulation in the usual manner. In the latter determinations, the areas of the zones of lysis were calculated and compared with those of control plates containing no EACA.

E. COAGULATION

Pooled rabbit plasma (10 mg of sodium citrate per ml of whole blood was used for all determinations. To 0.4 ml of plasma in 10 x 75 mm tubes was added 0.1 ml of buffer containing at least 10^8 cells (in qualitative assays) or decreasing amounts of cell extract or cellular debris (in quantitative assays). Tubes containing plasma plus borate buffer alone or plasma plus cells of a coagulase-positive strain of *Staphylococcus aureus* in borate buffer were always included as negative and positive controls, respectively. The tubes were incubated at 37 C and observed periodically for 48 hours; those showing a negative reaction at that time were assayed for the presence of the remaining fibrinogen by addition of excess Ca^{++}. This precaution proved necessary to avoid false-negative results caused by the action of F.

F. ADDITIONAL BACTERIAL PROPERTIES

Murine toxin (T) was determined qualitatively by intravenous injection of 100 μg of protein from cell-free extracts into each of 10 mice. This dose is uniformly fatal within 24 hours in the case of toxigenic extracts, but all animals receiving nontoxigenic extracts survive. The inability of cells grown at 26 C on slopes of BAB to remain in suspension upon storage at -23 C in stocks of glycerol-phosphate buffer was judged as presumptive evidence for the absence of antigen 4. The presence of antigens 4, T, fraction I and V was determined by gel diffusion.[20] Assays for PI and pesticin II (PII) were performed as previously described.[15]

Pigmentation (P) and calcium dependence (Ca) were determined on the medium of Jackson and Burrows[21] and Higuchi and Smith,[22] respectively. Abilities to ferment glycerol (Gly^+), rhamnose (Rha^+), and melibiose (Mel^+) were assayed with Difco purple broth containing 1% carbohydrate and 0.25% agar; production of urease (Ure) was determined with Christenson's urea agar (Difco).

G. UNITS OF ACTIVITY

One unit of PI, F, or C is defined as the least amount that yields detectable activity when assayed by the methods previously described. Specific activity is generally expressed in terms of volume to facilitate a direct comparison of ratios.

H. GEL FILTRATION

After hydration in borate buffer for 72 hours at room temperature, Sephadex G-200 was packed to a height of 230 mm in a glass column (20 x 300 mm). The bottom of the column, composed of highly porous sintered glass, was layered to a height of 1 mm with fine glass beads. A hydrophilic porous plastic disc* placed on the surface of the gel prevented distortion by the eluent (borate buffer). After the flow rate was adjusted to 15 ml per hour, 0.2 ml of cell-free extract was applied to the surface of the disc; fractions of 1 ml volume were collected at room temperature and assayed for PI, F, C, and protein; the latter was determined by the method of Lowry et al.[23]

I. REVERSION TO PESTICINOGENY

Suspensions of nonpesticinogenic cells of P. pestis were irradiated with ultraviolet light (UV) for a time sufficient to reduce viability to about 20% of that originally present. Surviving cells were then cultured overnight in HIB and their progeny plated on BAB at about 3,000 cells per petri dish. After incubation at 26 C for 48 hours, the colonies were treated with chloroform vapor and then overlayered with pesticin agar[16] seeded with a sensitive strain of P. pseudotuberculosis. Similar experiments were performed after growth for 4 days in a medium containing 5 mg of 2-aminopurine (AP) per ml of HIB.

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* Bel-Art Products, Pequannock, New Jersey.

J. REAGENTS AND NATURAL PRODUCTS

Plasma (10 mg of citrate per ml) and sera (rabbit, human, dog, sheep, and horse) were freshly obtained. Thrombin (bovine, topical) and fibrinogen (bovine fraction I containing 40 to 50% citrate) were obtained from Parke, Davis and Company and Armour and Company respectively. Recrystallized trypsin and EACA were products of the Nutritional Biochemicals Corporation, Cleveland, Ohio. AP was purchased from Calbiochem, Los Angeles, and plasminogen* was a product of the Cutter Laboratories, Berkeley, California.

III. RESULTS

A. COMPARISON OF PHENOTYPES

The phenotypes of wild-type P. pestis and P. pseudotuberculosis as well as of some atypical strains used in this study are shown in Table 1. There is no apparent correlation between expressions of the properties listed other than that previously noted for PI, F, and C.

B. DISTRIBUTION OF PI, F, AND C

Preliminary experiments showed that the activity of PI, F, and C in cellular debris was not altered by sterilization with chloroform vapor. Accordingly, surviving bacteria within the samples of cellular debris did not interfere with quantitative assays for PI, F, and C, and no attempt was made to sterilize these preparations for studies on anatomical distribution. As shown in Table 2, the ratio of PI to F in cell-free extracts is greater than that found for cellular debris. The activity of C was always lower than that of PI or F, a finding that may reflect the presence of inhibitors in plasma as well as a less sensitive method of assay. Nevertheless, it is clear that the ratios of PI to C are different for cell-free extracts and corresponding preparations of cellular debris. The ratios do not permit a distinction between F and C.

C. GEL FILTRATION

F from an extract of P. pestis strain A4 passed rapidly through Sephadex G-200, indicating that this substance is either a very large molecule or particulate (Fig. 1). In contrast, PI moved more slowly in the manner of a large soluble molecule. Although some C was present in the material added to the column, this activity could not be recovered, presumably because of dilution.

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* 1,000 RPMI (Roswell Park Memorial Institute) units per 100 mg.

TABLE 1. EXPRESSION OF SOME BACTERIAL PROPERTIES BY PESTICINOGENIC AND NONPESTICINOGENIC PASTEURELLAE

Strain[b]	Bacterial Property[a]												
	VW	Ca	P	Fl	4	T	Gly	Mel	Rha	Ure	PI	F	C
P. pestis													
Wild Type	+[c]	+	+	+	+	+	±	0	0	0	+	+	+
A4 (NP)	0	0	0	+	+	+	0	0	0	0	+	+	+
A4 (NP)X	0	0	0	+	+	+	0	0	0	0	0	0	0
A1122	0	0	0	NT	+	NT	0	0	0	0	+	+	+
A1122X	0	0	0	NT	+	NT	0	0	0	0	0	0	0
A12	0	0	+	+	0	+	0	0	0	0	0	0	0
Q14	0	0	+	+	+	+	0	0	0	0	+	+	+
B14	0	0	+	0	0	0	0	0	0	0	+	+	+
BC343	+	+	0	+	+	+	+	0	0	0	+	+	+
BC343X	+	+	0	+	+	+	+	0	0	0	0	0	0
DI	+	0	0	+	+	+	+	0	0	0	+	+	+
EV76	+	+	0	+	+	+	0	0	0	0	+	+	+
EV76X	+	+	0	+	+	+	0	0	0	0	0	0	0
G25	+	+	0	+	+	+	0	0	0	0	0	0	0
G32	+	+	+	+	+	+	0	0	0	0	0	0	0
G35	0	0	0	0	+	+	0	0	0	0	0	0	0
G50	0	0	0	0	+	0	+	0	0	0	0	0	0
Harbin	0	0	0	+	+	0	+	0	0	0	0	0	0
Java	0	0	0	0	+	0	0	0	0	0	0	0	0
TRU	0	0	0	0	+	0	0	0	0	0	0	0	0
URR	0	0	0	+	+	+	+	0	0	+	+	+	+
019	+	+	0	0	+	0	0	0	+	0	0	0	0
P. pseudotuberculosis													
Wild type	+	+	+	0	+	0	+	+	+	+	0	0	0

a. Phenotypic symbols are defined in the text.
b. Strains suffixed with an X were isolated from the corresponding prototroph listed immediately above.
c. + = positive, 0 = negative, ± = variable, and NT = not tested.

TABLE 2. DISTRIBUTION OF PESTICIN I (PI), FIBRINOLYTIC FACTOR (F), AND COAGULASE (C) IN WASHED CELLULAR DEBRIS AND CYTOPLASM OF PASTEURELLAE[a]

	Cellular Debris			Cytoplasm		
Strain	PI	F	C	PI	F	C
P. pestis						
A4	2.1×10^4	2.8×10^6	1.4×10^2	4.6×10^4	1.1×10^4	0
A4X	0	0	0	0	0	0
BC343	6.2×10^3	3.2×10^6	1.6×10^2	6.5×10^3	1.9×10^5	3×10^0
BC343X	0	0	0	0	0	0
A1122	3.9×10^3	4.9×10^5	2.4×10^1	6.5×10^3	5.1×10^4	10^0
A1122X	0	0	0	0	0	0
URR	2.1×10^3	1.4×10^6	1.1×10^2	5.4×10^3	2.2×10^4	10^0
P. pseudotuberculosis						
PB1/.	0	0	0	0	0	0

a. Values expressed as units per ml.

D. SENSITIVITY TO HEAT

The percentage of PI, F, and C in cellular debris remaining after boiling in tris buffer is shown in Figure 2. PI was lost rapidly during this treatment, but boiling for 15 minutes destroyed only 50% of the F and C. These findings again failed to show that molecules of F are distinct from those of C; the results are, however, in agreement with previous studies on heat lability of PI[11] and F.[24]

E. ACTIVATION OF PI, F, AND C

Cellular debris from the pesticinogenic strains A4 and A1122 was mixed and incubated at 26 and 37 C with equal volumes of extract from the non-pesticinogenic strains A4X and A1122X. Samples were removed at intervals over an 8-hour period and centrifuged; the supernatant was tested for PI. Similar experiments were performed with other combinations of samples, such as cellular debris of strains A4X and A1122X mixed with extracts of their pesticinogenic prototrophs or with pooled samples that had been freed of F or PI by gel filtration. In no case was appearance of new PI, F, or C detected, suggesting that these substances are not synthesized in a precursor state requiring a common activator for their expression or release.

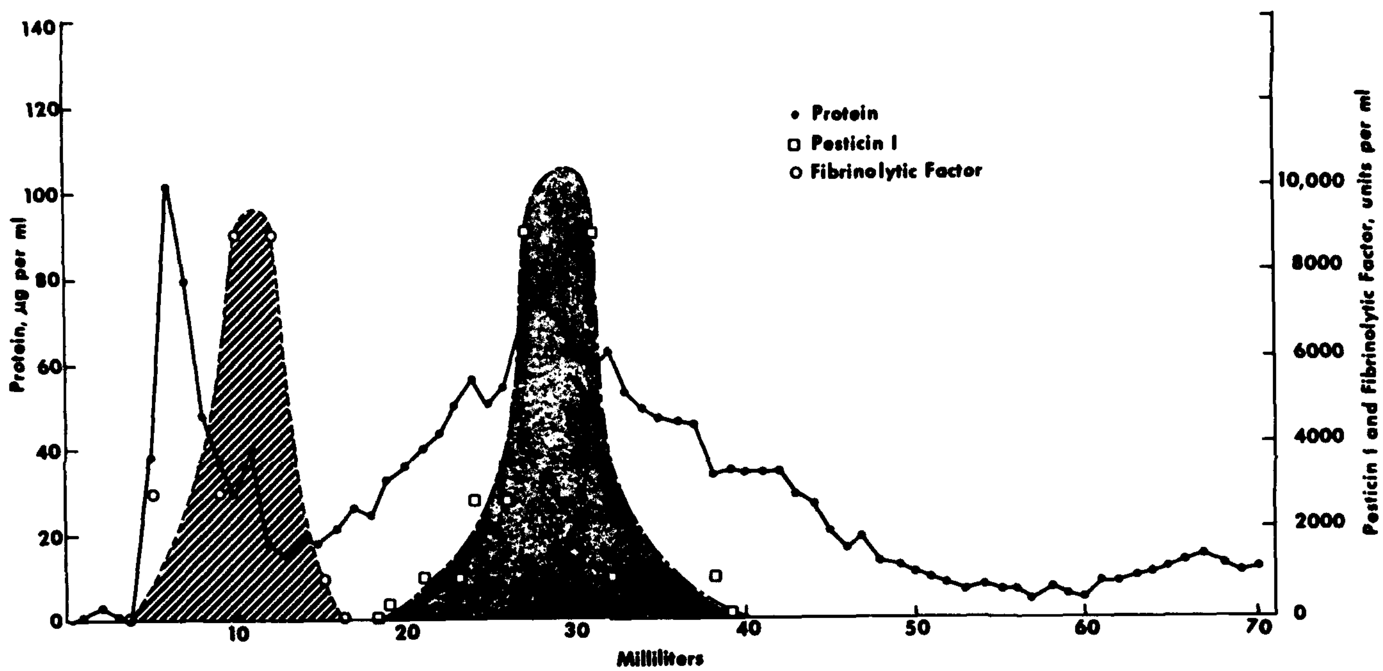

Figure 1. Recovery of Fibrinolytic Factor, Pesticin I, and Protein from Cytoplasm of P. pestis Strain A4 Following Filtration on Sephadex G-200.

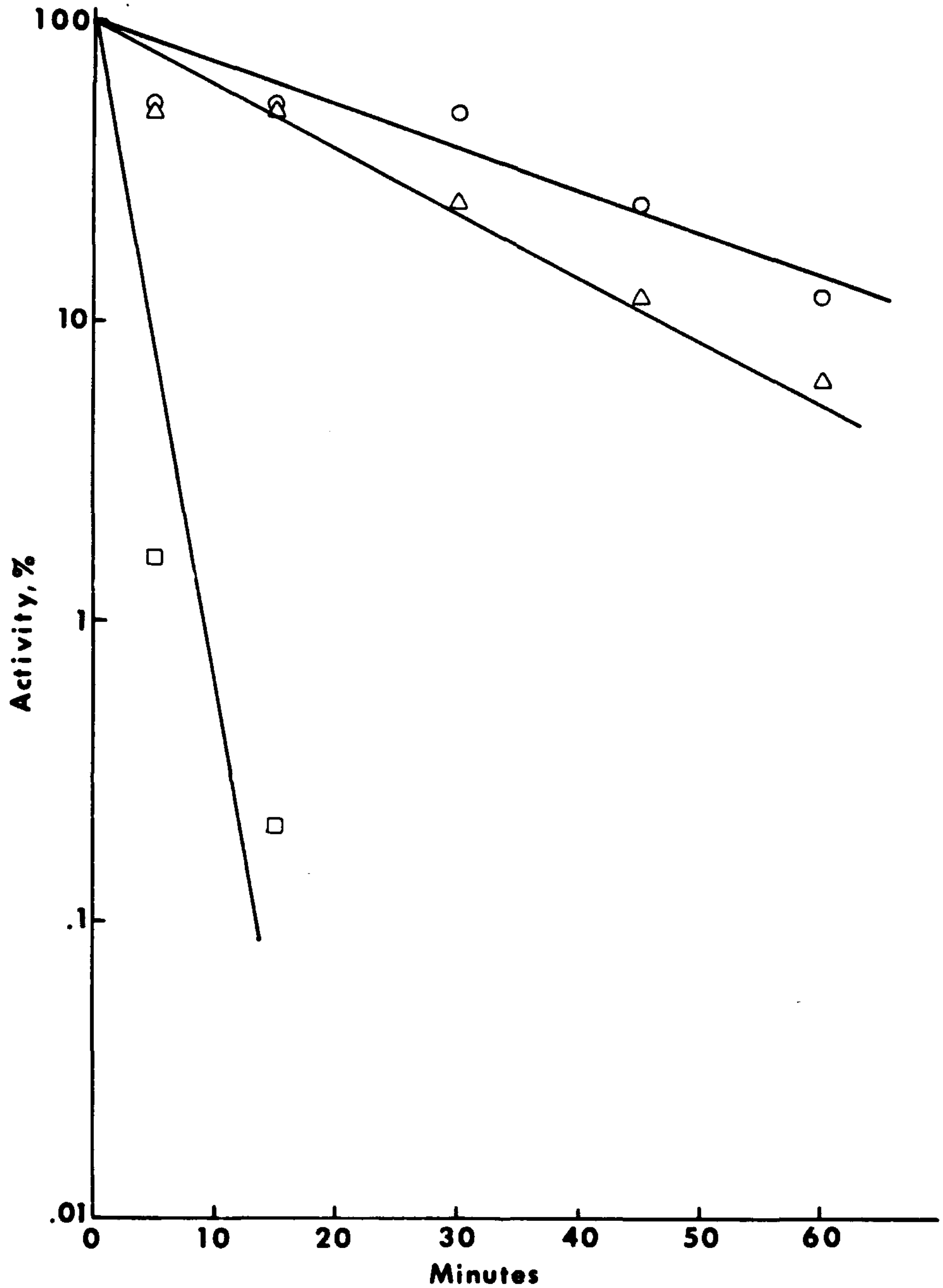

Figure 2. Destruction of Pesticin I (□), Fibrinolytic Factor (0), and Coagulase (△) during Boiling in 0.1 M Tris Buffer, pH 7.8.

F. TESTS FOR MUTATION TO PESTICINOGENY

P. pestis strains A4X and A1122X failed to yield pesticinogenic mutants either spontaneously or after treatment with UV or AP. Again, spontaneous or induced suppressor mutations or reversions permitting expression of PI, F, and C by strains G32 or A12 were not detected following growth at 37 C in HIB containing 1% calcium ethylenediaminetetraacetic acid (Ca-EDTA) and 0.01 M Ca^{++}. It should be noted that these two strains are sensitive to PI and that any pesticinogenic variants should have been selected under these cultural conditions.[13,15]

G. MECHANISM OF FIBRINOLYSIS

To distinguish between direct proteolysis and activation of plasminogen, F from cellular debris was titrated on normal and heated (plasminogen-free) fibrin films. Although 0.01 μg of cellular debris was capable of causing some lysis on unheated plates, activity on heated plates was never observed. In contrast, the end point for recrystallized trypsin was about 0.03 μg both on heated and on control films. However, fibrinolysis was observed on heated films when cellular debris was mixed with plasminogen of human origin (Fig. 3) or sera or plasma from five other mammalian species. These findings indicate that at least one heat-labile substance of host origin is necessary for the expression of F. Evidence suggesting that this substance is plasminogen was obtained by use of the inhibitor EACA.

According to Alkjaersig, Fletcher, and Sherry,[25] concentrations of EACA equal to or greater than 0.005 M noncompetitively inhibit the proteolytic action of plasmin although concentrations of 0.003 M or less competitively inhibit the activation of plasminogen by streptokinase or urokinase. Fibrin films containing decreasing amounts of EACA were spotted in triplicate with cellular debris containing F. After fibrinolysis, average zone sizes were calculated and compared with those produced on films containing no EACA. As shown in Table 3, decreased areas of fibrinolysis were always obtained in the presence of 0.05 or 0.025 M EACA, a result that presumably reflects inhibition of both F and plasmin. However, F in low concentrations was significantly inhibited by 0.003 M EACA, indicating a lytic mechanism analogous to that of urokinase or staphylokinase. The stimulatory effect of small concentrations of EACA probably reflects its ability to enhance the activity of plasmin.[25]

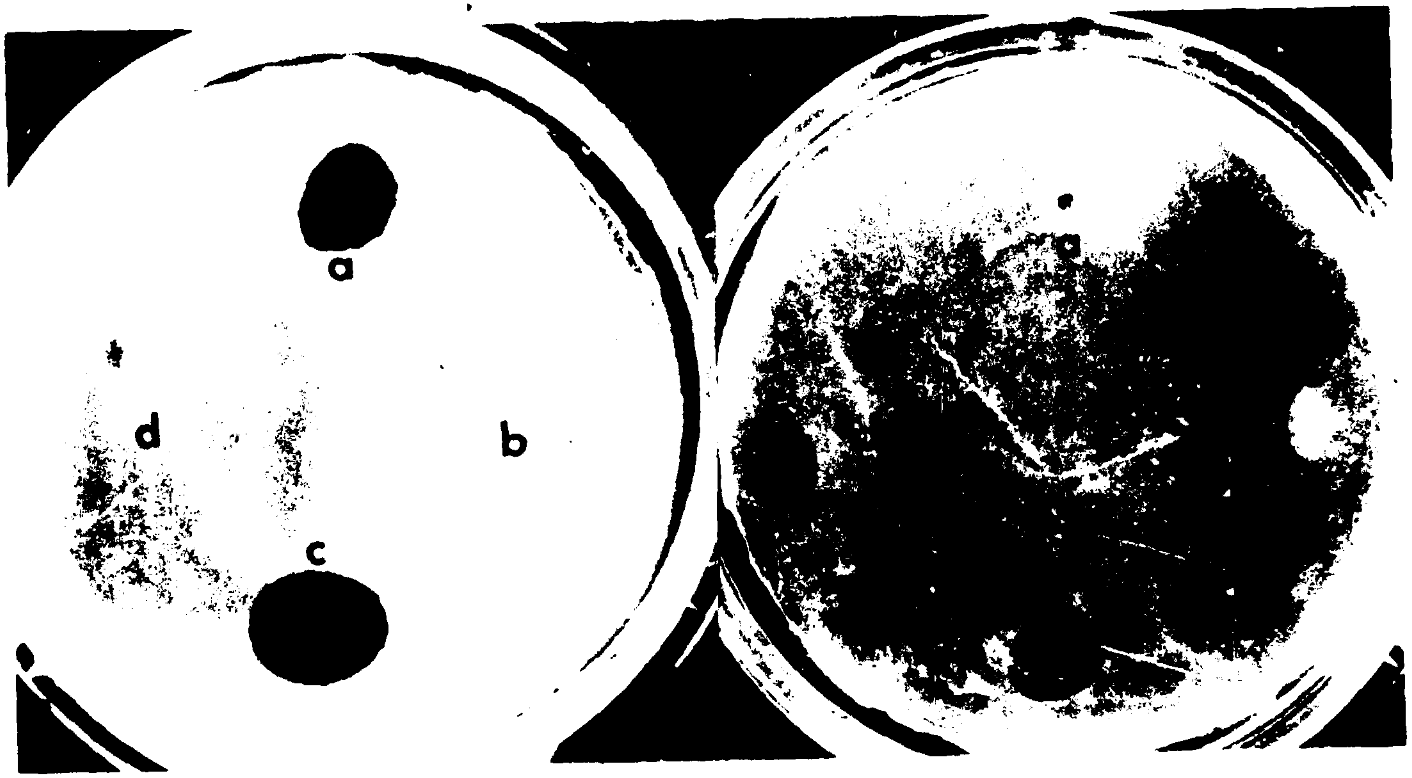

Figure 3. Comparative Fibrinolytic Activity of P. pestis Cellular Debris on Heated (Right) and Unheated (Left) Bovine Fibrin Films. (a) 8 μg debris + borate buffer, (b) 0.01 RPMI units of human plasminogen + borate buffer, (c) 8 μg debris + 0.01 RPMI unit of human plasminogen, and (d) borate buffer.

TABLE 3. PER CENT INHIBITION OF FIBRINOLYSIN BY VARIOUS AMOUNTS OF ε-AMINOCAPROIC ACID INCORPORATED DIRECTLY INTO FIBRIN FILMS

Cellular Debris, μg	ε-Aminocaproic Acid, mM per liter						
	50	25	12.5	6.2	3.1	1.5	0.8
20	63	39	48	39	0	11	S[a]
10	56	63	48	39	0	11	0
5	69	48	56	48	39	21	21
2.5	43	30	16	30	S	S	S
1.25	52	52	19	19	S	S	S
0.62	56	41	22	0	S	S	S
0.31	PL[b]	44	44	44	S	S	S
0.16	100	PL	44	74	34	S	34
0.08	100	100	100	PL	75	0	39
0.04	100	100	100	100	PL	PL	9

a. S = Stimulation of fibrinolysis (zones of fibrinolysis on plates containing ε-aminocaproic acid being somewhat larger than those on control plates lacking the inhibitor).

b. PL = Partial lysis (small, ill-defined areas of lysis).

IV. DISCUSSION

Prior to this investigation, at least four mutational events had to be considered as possible explanations for the single-step concomitant loss of PI, F, and C: (i), if these three activities are functions of the same protein or polypeptide, then they would be lost following alteration or loss of the corresponding gene; (ii), if PI, F, C, or some fourth substance is required as a common activator, mutational loss of this activator would accomplish the same result; (iii), alteration or loss of a common operator or regulator gene could conceivably result in loss of the three activities; (iv), structural genes for three activities could exist on a replicon distinct from the chromosome proper, and loss of this replicon would then result in loss of PI, F, and C. A direct determination of the correct alternative cannot, of course, be made until an appropriate method of genetic transfer is developed for P. pestis. Nevertheless, some of the possibilities just listed can be discounted.

Although F and C could not be distinguished from one another except by biological assay, the differences in cellular distribution, stability toward heat, and behavior on Sephadex G-200 showed clearly that these two activities cannot be equated with those of PI. This finding indicates that at least two genes are involved in expression of the three activities, and the first possibility must be discarded. The second alternative also seems unlikely because of repeated failure to demonstrate an activator of PI, F, and C analogous, say, to that system known to activate plasminogen. The chance always exists that such activation may be detected under different experimental conditions, but regulation at this level is rare in bacteria and probably does not occur with respect to PI, F, and C.

The third alternative implies that the expression of structural genes for PI, F, and C requires the presence of a functional operator or regulator gene,[26] which is lost or altered in the typical nonpesticinogenic mutant. If this notion is correct, then suppressor mutations or reversions to prototrophy might occur in nonpesticinogenic organisms; such reversion has not yet been observed by us or reported by others. Accordingly, no evidence could be obtained that discredits the fourth alternative, which proposes the existence of linked structural genes for the three activities on an extrachromosomal determinant.

These findings were not unexpected in view of the fact that PI possesses certain properties common to known bacteriocins[11,12] that are generally controlled by extrachromosomal determinants termed plasmids.[27] However, the nature of PI was left in some doubt upon finding that this substance differed from many bacteriocins in possessing a broad indicator range,[13,14] was inhibited by Fe^{+++} and hemin,[13] and exhibited properties of a constitutive metabolite.[15] The data presented here probably represent the best evidence available for consideration of PI as a true bacteriocin. These studies also point out the difficulties inherent in attempting genetic analysis of an organism not amenable to controlled chromosomal transfer.

No evidence was obtained showing that F and C are distinct chemical entities. It cannot be assumed that these two activities are functions of the same molecule, but it should be noted that under certain conditions crystalline trypsin is said to activate both plasminogen and prothrombin and thus act as fibrinolysin and coagulase, respectively.[28,29] A correlation between expression of fibrinolytic and coagulase activities has also been noted in other biological systems. For example, Kapral and Li[30] selected coagulase-negative mutants of Staphylococcus aureus that subsequently were found to lack staphylokinase. These isolates were presumed to represent double mutants, but the possibility of a single event was not disproved.

The F of P. pestis did not attack fibrin directly as do the fibrinolysins of certain species of Clostridia.[31] Instead, F resembled staphylokinase or urokinase, enzymes that are known activators of plasminogen.[29,32,33] No requirement was found for a host-specific proactivator such as that needed for maximum activity of streptokinase.[34] No attempt was made to show that F catalyzes an enzymatic conversion of plasminogen to plasmin, but the fact that only 0.01 μg of cellular debris was necessary for activity suggests that this is the case.

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13. ABSTRACT

Mutational loss of pesticin I, a bacteriocin-like substance produced by Pasteurella pestis, results in concomitant loss of a coagulase and a fibrinolytic factor. No relationship was detected between pesticinogeny and other tested properties either associated with virulence or peculiar to P. pestis. Pesticin I was distinguished from the coagulase and fibrinolytic activities on the basis of anatomical distribution, behavior during gel filtration, and sensitivity to heat. Coagulase and the fibrinolytic factor were not differentiated by these criteria. Spontaneous suppressor mutations causing reversion to pesticinogeny were not detected, nor were such mutants obtained by treatment with ultraviolet light or 2-aminopurine. Attempts to demonstrate a common activator of pesticin I, coagulase, or the fibrinolytic factor in extracts of pesticinogenic cells were not successful. These results are in accord with the hypothesis that at least two structural genes for the three activities reside on a replicon distinct from the chromosome proper. Fibrinolytic activity was significantly reduced in the presence of 0.003 M ϵ-aminocaproic acid and was nonexistent on fibrin films freed of endogenous plasminogen by treatment with heat. Fibrinolytic activity on heated films could be restored by addition of plasma or serum from six mammalian species. Accordingly, the plague fibrinolytic factor, like staphylokinase or urokinase, promotes the conversion of plasminogen to plasmin.

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